Claims

1. A method for determining an amino acid sequence binding motif for a phosphorylation site of a kinase, comprising:

a) contacting the kinase with a peptide library, wherein each peptide comprises a single non-degenerate phosphorylatable amino acid in a fixed position of the peptide and wherein each peptide comprises one or more degenerate amino acids, under conditions which allow for binding of a peptide by the kinase at the phosphorylation site of the kinase;

b) allowing the kinase to bind peptides of the peptide library having a binding site for the kinase phosphorylation site to form kinase-peptide complexes;

c) isolating the kinase-peptide complexes from the unbound peptides;

d) releasing the peptides from the kinase-peptide complexes;

e) isolating the peptides previously bound to the kinase in kinase-peptide complexes;

f) determining the amino acid sequences of the peptides isolated in (e); and

g) determining an amino acid sequence motif for a binding site of the kinase based upon the relative abundance of different amino acid residues at each degenerate position within the peptides.

2. The method of claim 1, wherein the peptide library comprises peptides comprising the formula:

$$(Xaa)_n\text{-}Zaa\text{-}(Xaa)_m \quad \text{(SEQ ID NO:13)}$$

wherein Zaa is the single non-degenerate phosphorylatable amino acid and is selected from the group consisting of Tyr, Ser and Thr,

wherein Xaa is any amino acid except Zaa, and

wherein n and m are integers from 1-10 inclusive.

3. The method of claim 1, wherein the peptide library is a soluble synthetic peptide library.

4. The method of claim 1, wherein the single non-degenerate phosphorylatable amino acid is tyrosine.

5. The method of claim 4, wherein the peptides comprise the amino acid sequence Xaa_n-Tyr-Xaa_m (SEQ ID NO:14), wherein Xaa is any amino acid except Tyr and wherein n and m are integers from 1-10 inclusive.

6. The method of claim 4, wherein the peptides comprise the amino acid sequence Xaa_n-Tyr-Xaa_m (SEQ ID NO:15), wherein Xaa is any amino acid except Tyr or Cys and wherein n and m are integers from 1-10 inclusive.

7. The method of claim 4, wherein the peptides comprise the amino acid sequence Xaa_n-Tyr-Xaa_m (SEQ ID NO:16), wherein Xaa is any amino acid except Tyr, Cys or Trp and wherein n and m are integers from 1-10 inclusive.

8. The method of claim 7, wherein the peptides comprise the amino acid sequence Xaa_4-Tyr-Xaa_4 (SEQ ID NO:17), wherein Xaa is any amino acid except Tyr, Cys or Trp .

9. The method of claim 1, wherein the single non-degenerate phosphorylatable amino acid is serine.

10. The method of claim 9, wherein the peptides comprise the amino acid sequence Xaa_n-Ser-Xaa_m (SEQ ID NO:18), wherein Xaa is any amino acid except Ser and wherein n and m are integers from 1-10 inclusive.

11. The method of claim 9, wherein the peptides comprise the amino acid sequence Xaa_n-Ser-Xaa_m (SEQ ID NO:19), wherein Xaa is any amino acid except Ser or Cys and wherein n and m are integers from 1-10 inclusive.

12. The method of claim 9, wherein the peptides comprise the amino acid sequence Xaa_n-Ser-Xaa_m (SEQ ID NO:20), wherein Xaa is any amino acid except Ser, Cys or Trp and wherein n and m are integers from 1-10 inclusive.

13. The method of claim 1, wherein the single non-degenerate phosphorylatable amino acid is threonine.

14. The method of claim 13, wherein the peptides comprise the amino acid sequence Xaa_n-Thr-Xaa_m (SEQ ID NO:21), wherein Xaa is any amino acid except Thr and wherein n and m are integers from 1-10 inclusive.

15. The method of claim 13, wherein the peptides comprise the amino acid sequence Xaa_n-Thr-Xaa_m (SEQ ID NO:22), wherein Xaa is any amino acid except Thr or Cys and wherein n and m are integers from 1-10 inclusive.

16. The method of claim 13, wherein the peptides comprise the amino acid sequence Xaa_n-Thr-Xaa_m (SEQ ID NO:23), wherein Xaa is any amino acid except Thr, Cys or Trp and wherein n and m are integers from 1-10 inclusive.

17. The method of claim 1, wherein the peptide library is contacted with the kinase by application of the library to a substrate to which the kinase is immobilized.

18. The method of claim 1, wherein the kinase-peptide complexes are isolated by washing the kinase-peptide complexes in a buffer that permits binding of peptides to the phosphorylation site of the kinase.

19. The method of claim 1, wherein the peptides are eluted from the kinase-peptide complexes by incubating the kinase-peptide complexes with an elution solution.

20. The method of claim 19, wherein the elution solution has an acidic pH.

21. A kinase binding molecule comprising a binding motif for a phosphorylation site of a kinase identified according to any of claims 1-20.

22. The kinase binding molecule of claim 21, further comprising a molecule that mediates transport across a plasma membrane.

23. The kinase binding molecule of claim 22, wherein the molecule that mediates transport across a plasma membrane is selected from the group consisting of penetratin, Tat, VP22, Pep-1 and fragments thereof that mediate transport across a plasma membrane.

24. A composition comprising the kinase binding molecule of claim 21 and a pharmaceutically acceptable carrier.

25. Use of the kinase binding molecule of claim 21 in the preparation of a medicament.

26. A method for inhibiting phosphorylation of proteins by a kinase, comprising, contacting the kinase with an amount of the kinase binding molecule of any of claims 21-23 or the composition of claim 24 effective to inhibit the phosphorylation.

27. A method for treating a condition that includes phosphorylation of proteins by a kinase, comprising,
administering to a subject an amount of the kinase binding molecule of any of claims 21-23 or the composition of claim 24 effective to inhibit the phosphorylation of the proteins by the kinase.

28. The method of claim 27, wherein the subject is a mammal.

29. The method of claim 27, wherein the subject is a human.

30. A kinase inhibitor comprising a binding motif for a phosphorylation site of a kinase identified according to any of claims 1-20, wherein the single non-degenerate phosphorylatable amino acid is replaced by an amino acid that cannot be phosphorylated by the kinase to which the inhibitor binds.

31. The kinase inhibitor of claim 30, wherein the binding motif comprises SEQ ID NO:2.

32. The kinase inhibitor of claim 30, wherein the binding motif comprises SEQ ID NO:4.

33. The kinase inhibitor of claim 31 or 32, wherein the amino acid that cannot be phosphorylated is Tyr and the Tyr is replaced by Phe or a halogenated Phe.

34. The kinase inhibitor of any of claims 30-33, further comprising a molecule that mediates transport across a plasma membrane.

35. The kinase inhibitor of claim 34, wherein the molecule that mediates transport across a plasma membrane is selected from the group consisting of penetratin, Tat, VP22, Pep-1 and fragments thereof that mediate transport across a plasma membrane.

36. A composition comprising the kinase inhibitor of any of claims 30-33 and a pharmaceutically acceptable carrier.

37. Use of the kinase inhibitor of claim 30 in the preparation of a medicament.

38. A method for inhibiting phosphorylation of proteins by a kinase, comprising,

contacting the kinase with an amount of the kinase inhibitor of any of claims 30-35 or the composition of claim 36 effective to inhibit the phosphorylation.

39. A method for treating a condition that includes phosphorylation of proteins by a kinase, comprising,

administering to a subject an amount of the kinase inhibitor of any of claims 30-35 or the composition of claim 36 effective to inhibit the phosphorylation of the proteins by the kinase.

40. The method of claim 39, wherein the subject is a mammal.

41. The method of claim 40, wherein the subject is a human.

42. A method for validating a kinase as a target for inhibition for the treatment of a condition, comprising

providing a molecule comprising a binding motif for a phosphorylation site of a kinase as claimed in any of claims 1-20,

contacting a biological sample containing a kinase suspected of being involved in the causation of the condition with the molecule for a time sufficient to permit binding of the molecule and the kinase, and

determining the effect of the molecule on one or more biological processes mediated by the kinase.

43. The method of claim 42, wherein the biological sample is a cell.

44. The method of claim 42, wherein the biological sample is an organism.

45. The method of claim 43 or 44, wherein the cell or organism is a model system for the condition.

46. A method for inhibiting a ZAP-70 kinase comprising,

contacting the ZAP-70 kinase with an amount of a kinase inhibitor as claimed in any of claims 30-35, effective to inhibit the ZAP-70 kinase.

47. The method of claim 46, wherein the kinase inhibitor comprises SEQ ID NO:4 or SEQ ID NO:9.

48. A method for treating a condition mediated by a ZAP-70 kinase comprising

administering to a subject in need of such treatment an amount of a kinase inhibitor as claimed in any of claims 30-35 or a composition as claimed in claim 36 , effective to inhibit the ZAP-70 kinase.

49. The method of claim 48, wherein the kinase inhibitor comprises SEQ ID NO:4 or SEQ ID NO:9.

50. The method of claim 49, wherein the subject is a mammal.

51. The method of claim 50, wherein the subject is a human.

52. A method for inhibiting transcription mediated by a ZAP-70-responsive promoter sequence, comprising

contacting a biological sample, cell or organism that comprises a ZAP-70-responsive promoter sequence operably linked to a nucleic acid molecule with an amount of a kinase inhibitor as claimed in any of claims 30-35 effective to inhibit the transcription of the nucleic acid molecule mediated by the ZAP-70-responsive promoter sequence.

53. The method of claim 52, wherein the kinase inhibitor comprises SEQ ID NO:4 or SEQ ID NO:9.

54. The method of claim 52, wherein the ZAP-70-responsive promoter sequence is an interleukin-2 promoter.

55. A method for treating a condition mediated by transcription mediated by a ZAP-70-responsive promoter sequence, comprising

administering to a subject in need of such treatment an amount of a kinase inhibitor as claimed in any of claims 30-35 or a composition as claimed in claim 36 , effective to inhibit the transcription mediated by the ZAP-70-responsive promoter sequence.

56. The method of claim 55, wherein the kinase inhibitor comprises SEQ ID NO:4 or SEQ ID NO:9.

57. The method of claim 55, wherein the ZAP-70-responsive promoter sequence is an interleukin-2 promoter.

58. The method of claim 55, wherein the subject is a mammal.

59. The method of claim 58, wherein the subject is a human.

60. A method for identifying a kinase inhibitor compound, comprising

providing a kinase, a kinase inhibitor that binds the kinase, and a candidate kinase inhibitor compound,

contacting the kinase with the candidate kinase inhibitor compound and the kinase inhibitor under conditions that permit binding of the kinase inhibitor to the kinase, wherein either or both of the candidate kinase inhibitor compound and the kinase inhibitor are detectable, and wherein either or both of the candidate kinase inhibitor compound and the kinase inhibitor comprises a sequence determined according to any of claims 1-20,

separating the kinase from the unbound kinase inhibitor and unbound candidate kinase inhibitor compound, and

detecting the amounts of detectable kinase inhibitor and/or the detectable candidate kinase inhibitor compound bound to the kinase as a measure of the presence of a candidate kinase inhibitor compound that competes with the kinase inhibitor for binding to the kinase.

61. The method of claim 60, further comprising testing the activity of the kinase in the presence of the candidate kinase inhibitor compound, wherein a greater reduction in kinase activity in the presence of the candidate kinase inhibitor compound than in the absence of the candidate kinase inhibitor compound indicates that the candidate kinase inhibitor compound is a kinase inhibitor.

62. The method of claim 60, wherein the candidate kinase inhibitor compound or the kinase inhibitor comprises a binding motif sequence comprising SEQ ID NO:13.

63. The method of claim 60, wherein the candidate kinase inhibitor compound is a small organic molecule.

64. A kinase inhibitor compound identified according to any one of claims 60-63.

65. Use of the kinase inhibitor compound of claim 64 in the preparation of a medicament.